Lipid Phase Perturbations and the Unfolded Protein Response

Recent studies of the consequences of ganglioside accumulation in lysosomal storage disease and free cholesterol accumulation in cell membranes in atherosclerosis suggest an unexpected link between perturbation of the endoplasmic reticulum membrane's lipid phase, induction of the unfolded protein response, and cell death.

Johann Thudicum, the discoverer of sphingolipids, named them after the Sphinx of Egypt, to emphasize their enigmatic nature. One hundred years later, their physiological role remains rather obscure; however, a paper in the September 10th issue of *Molecular Cell* (Tessitore et al., 2004) provides new insight into the pathogenesis of a disorder associated with abnormal accumulation of a sphingolipid, G_{M1} gangliosidosis.

G_{M1} gangliosides are major constituents of the outer leaflet of the plasma membrane, accounting for 5%–10% of the lipid mass of the plasma membrane of neurons. Their biosynthesis takes place in the Golgi apparatus and their turnover is mostly a lysosomal affair, executed by specific hexosidases that remove the complex sugars from the sphingolipid core. Mutations in the lysosomal β-galactosidase (β-gal) are associated with abnormal accumulation of G_{M1} and lead to a progressive neurodegenerative disorder. Abnormal lysosomes are the conspicuous morphological feature of G_{M1} gangliosidosis, and from this perspective the disease resembles many other lysosomal storage diseases, which often have conspicuous neurological phenotypes. Despite being well characterized morphologically and biochemically, the pathophysiology of G_{M1} gangliosidosis (and other lysosomal storage diseases) is poorly understood.

Knockout mice for lysosomal β-gal provide a model system for human G_{M1} gangliosidosis, as they too manifest a progressive neurological disorder, lysosomal abnormalities, and intracellular accumulation of G_{M1} and other sphingolipids (Itoh et al., 2001). Tessitore and colleagues now report on previously overlooked abnormalities affecting the morphology of the endoplasmic reticulum (ER) in the brain of these mice (Tessitore et al., 2004). This finding presumably surprised them, as the metabolism of sphingolipids is believed to take place in post-ER compartments of the endomembrane system. However, they decided to pursue it because of an emerging link between ER stress and neurodegeneration (Kaufman, 2002) a link that could be relevant to the G_{M1} gangliosidosis.

Most secreted, lumenal, and membrane proteins are synthesized on the membranes of the rough ER. These nascent client proteins (or segments thereof) are translocated into the lumen of the organelle where specific enzymes and chaperones process, fold, and assemble them into functional complexes. Specific ER proteins (IRE1, PERK, and ATF6) monitor the balance between the load of unfolded client proteins presented to the ER and the capacity of the organelle to deal with that load (Mori, 2000). Imbalance, referred to as ER stress, activates the aforementioned stress receptors and their downstream effectors. Because it can be readily induced by perturbations of ER function that affect client protein folding, the ensuing response is referred to as the unfolded protein response (UPR). The UPR seeks to restore homeostasis by repressing synthesis of ER client proteins and by upregulating genes that function at all levels of client protein metabolism (Patil and Walter, 2001).

Toxins such as tunicamycin that selectively affect protein folding in the ER kill cells. Furthermore, accumulation of malfolded proteins in the endoplasmic reticulum likely promotes cell death by certain toxic gain-of-function mutations that affect the folding or oligomerization of abundantly expressed ER client proteins (Kopito and Ron, 2000). Finally, loss-of-function mutations in components that signal the UPR markedly degrade viability of secretory cells (Harding et al., 2001). All these point to the potential adverse consequences of ER stress and to an important homeostatic role of the UPR.

In addition to the aforementioned toxins and relatively rare genetic disorders, ER stress seems to accompany more common conditions, such as ischemia and Parkinson's disease (Kumar et al., 2001; Ryu et al., 2002), though its role in their pathogenesis remains largely unexplored. The study in *Molecular Cell* shows that UPR markers are activated in brains of β-gal knockout mice and that elimination of β-4-*N*-acetylgalatosaminyltransferase, an enzyme required for G_{M1} synthesis, forestalls the UPR. Furthermore loading of cultured cells and neurospheres with G_{M1} can recapitulate UPR activation of the β-gal knockout mice. These observations implicate G_{M1} accumulation in causing ER stress. A plausible mechanism is provided by the observation that excess G_{M1} colocalizes with an ER marker and by the finding that G_{M1} loading diminishes ER calcium stores. That latter can directly account for development of ER stress, as many of the enzymes and chaperones that metabolize and fold ER client proteins depend on high lumenal concentrations of the divalent cation.

All this is reminiscent of observations made recently in cholesterol-loaded macrophages, where inhibitors of intracellular cholesterol trafficking and (re)esterification were used to support the hypothesis that abnormal accumulation of free cholesterol in the normally cholesterol-poor ER membrane also causes ER stress and depletes lumenal calcium stores (Feng et al., 2003). Though not proven, it is assumed that G_{M1} staining of the ER in the loaded fibroblasts and excess free cholesterol in the macrophages reflect their accumulation within the lipid bilayer. Taken together, these observations suggest that these abnormal lipid constituents perturb some aspect of ER membrane function; perhaps they promote a calcium leak from the ER or impair calcium re-uptake from the cytoplasm by inhibiting SERCA pumps. But other possibilities exist; for example, the neurons of the β-gal knockout mouse show extensive evidence of autophagy (Tessitore et al., 2004), which might erode ER mass and contribute to reduced calcium stores.

The new study raises interesting questions and provides insight into possible avenues for therapeutic intervention. It implies retrograde transport of sphingolipids from post-ER compartments to the ER, whose mechanism remains to be discovered. A better understanding of this retrograde transport process might uncover opportunities for interference, which could be of therapeutic benefit if G_{M1} accumulation in the ER membrane plays a role in pathogenesis. It is important, however, to emphasize that the role of ER stress in death of neurons in G_{M1} gangliosidosis, while plausible, remains unproven. Our ignorance regarding the mechanisms by which ER stress kills cells precludes strong experiments to test its role in pathophysiology, as knockouts of the few genes implicated in ER stress-mediated cell death have relatively modest affects. Finally, the biophysics of the hypothesized perturbation in the lipid phase of the ER membrane needs to be worked out, and this may bring about a better understanding of membrane specialization in eukaryotic cells.

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Selected Reading

Feng, B., Yao, P.M., Li, Y., Devlin, C.M., Zhang, D., Harding, H.P., Sweeney, M., Rong, J.X., Kuriakose, G., Fisher, E.A., et al. (2003). Nat. Cell Biol. *5*, 781–792.

Harding, H., Zeng, H., Zhang, Y., Jungreis, R., Chung, P., Plesken, H., Sabatini, D., and Ron, D. (2001). Mol. Cell *7*, 1153–1163.

Itoh, M., Matsuda, J., Suzuki, O., Ogura, A., Oshima, A., Tai, T., Suzuki, Y., and Takashima, S. (2001). Brain Dev. *23*, 379–384.

Kaufman, R.J. (2002). J. Clin. Invest. *110*, 1389–1398.

Kopito, R.R., and Ron, D. (2000). Nat. Cell Biol. *2*, E207–E209.

Kumar, R., Azam, S., Sullivan, J., Owen, C., Cavener, D., Zhang, P., Ron, D., Harding, H., Chen, J., Han, A., et al. (2001). J. Neurochem. *77*, 1418–1421.

Mori, K. (2000). Cell *101*, 451–454.

Patil, C., and Walter, P. (2001). Curr. Opin. Cell Biol. *13*, 349–355.

Ryu, E.J., Harding, H.P., Angelastro, J.M., Vitolo, O.V., Ron, D., and Greene, L.A. (2002). J. Neurosci. *22*, 10690–10698.

Tessitore, A., Martin, M.P., Sano, R., Ma, Y., Mann, L., Ingrassia, A., Laywell, E.D., Steindler, D.A., Hendershot, L.M., and d'Azzo, A. (2004). Mol. Cell *15*, 753–766.

Re*FUS*ing to Grow Up

At germination, plants must coordinate the exit from dormancy and the start of vegetative growth. In this issue of *Developmental Cell*, Gazzarrini et al. show that *FUSCA3* plays a critical role in regulating hormone levels to synchronize the transition from embryonic to vegetative growth in *Arabidopsis*.

Plant development requires the ordered expression of genetic programs that control the embryonic, juvenile, adult, and reproductive phases of growth. Over time, these programs alter the patterns of cell division, elongation, and specialization to create characteristic changes in plant morphology. These may include changes in phyllotaxy, internode length, and the size, shape, or identity of lateral organs (Poethig, 2003).

The extensive morphological changes that can result from shifts in the relative timing of developmental events (heterochronic shifts) demonstrate the importance of coordinating temporal programs. During plant development, the gradual progression from the embryonic to the reproductive stage does not appear to be under the control of a single "clock." In maize, for example, the switch from juvenile to adult development requires both the repression of the juvenile program and the activation of the adult program, and these events can be uncoupled by mutations such as *Teopod* (Poethig, 1988). Thus, phase transitions may require the simultaneous cessation and activation of two separate developmental programs.

A similar phenomenon is observed during embryogenesis, when the processes of morphogenesis, maturation, and germination must be coordinately regulated. Upon completing morphogenesis, *Arabidopsis* embryos enter a phase of dormancy that is induced by high levels of abscisic acid (ABA). At germination, increasing levels of gibberellin (GA) release the embryo from dormancy and promote shoot and root growth. Embryos deficient in ABA can undergo GA-independent germination (Bentsink and Koornneef, 2002), but still require GA to promote the expression of postembryonic traits, such as trichomes. Thus, in wild-type plants, the dual functions of GA help to coordinate the simultaneous repression of dormancy and initiation of vegetative growth. As with the juvenile to adult transition, however, it has been possible to isolate to isolate a number of mutations that disrupt this switch and cause the overlapping expression of embryonic and postembryonic programs (Meinke et al., 1994).

FUSCA3 (FUS3) was first identified as a loss-of-function mutation that causes the precocious expression of postembryonic traits: cotyledons develop adult leaf traits, dormancy is bypassed, and leaf-specific genes are prematurely derepressed. The heterochronic phenotype of *fus3* can be suppressed by exogenous ABA and compounds that inhibit GA synthesis, suggesting that these hormones act downstream of *FUS3* (Keith et al., 1994). In this issue of *Developmental Cell*, Gazzarrini et al. (2004) continue to explore the interactions of *FUS3*,